IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Sommermeyer et al.
Serial Number: 07/533,294
Filed: June 5, 1990
For: HYDROXYETHYLSTARCH (HES) AS PLASMAEXPANDER AND PROCESS FOR PREPARING HES

DECLARATION UNDER 37 CRF 1.132

Commissioner of Patent and
Trademark Office
Washington, DC 20036

Sir:

I, Dr. Klaus Sommermeyer, of Kapersburgstraße 6b, D-6365 Rosbach v.d.H., Federal Republic of Germany, declare and say as follows:

1. I received the degree of PHD in physical chemistry in the year 1976 from University of Freiburg/Germany.

2. I performed or supervised the experimental tests described below. A hydroxyethyl starch ("HES") plasma extender was prepared according to the protocol set forth in U.S. patent application Serial Number 07/533,294 of Sommermeyer et al. The product was an HES (500/03) derived from starch rich in amylopectin having molecular weight of 500,000 daltons; MS=0.30; DS=0.28; and C2/C6=8.5.

3. 500 ml of a 10 % solution of this plasma extender was administered by infusion to each of four male volunteers not having disturbed metabolism. The age of the volunteers was from 20 to 40 years.

4. The concentration of the HES in serum was determined at different times by the following assay:

Sample preparation:
10 ml of blood were drawn with a "Heparin-Monovette" and this blood sample was centrifugated at 3.000 rpm for 10 min. 4 ml of the supernatant were removed and filled into a centrifugal tube which contained 1.5 ml ICA-solution (50 %).

The sample was vibrated, thereafter centrifugated at 3.000 rpm for 10 min and then stored at -20°C.

For further processing the sample was thawed, centrifugated at 4.500 rpm for 15 min (temperature: +4°C; 9 accel; 5 decel), quantitatively filled into a 100 ml centrifugal tube and then 60 ml of acetone were added to this sample.

The precipitated HES was deposited by direct centrifugation (4.500 rpm; 15 min; temperature 4°C; 9 accel; 5 decel). The supernatant acetone was decanted.

When the HES concentration in the blood is low, a concentration can be made by further sample material to the residue according to the given description.

The centrifugal tube was blown out with nitrogen (N_2) and then the residue was dissolved in 2.5 ml sodium azide solution (0.03 %).

Quantitative evaluation of HES:

The HES sample isolated according to the above sample preparation was analysed in an analogous manner as can be seen from the enclosed article "Studies on Hydroxyethyl Starch" (Arzneim. Forsch./Drug. Res. 35 (1), Nr. 3 (1985), pages 615-622) by comparing the area values of the RI signal with the area values of a reference sample.

The assay results are illustrated in Figure 1.

5. From the assay results, I conclude that HES (500/03) plasma extender was substantially eliminated from the plasma of the volunteers within from about three hundred sixty to four hundred eighty minutes.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FURTHER DECLARANT SAYETH NOT

6.12.97
Date

Dr. Klaus Sommermeyer